Customer No.	026418	
Attorney's Docket No.:	GK-ZEI-3130 / 500343.20130	
U.S. Application No.:	09/869951	
International Application No.:	PCT/EP00/10808	
International Filing Date:	NOVEMBER 02, 2000	02 NOVEMBER 2000
Priority Date Claimed:	NOVEMBER 10, 1999	10 NOVEMBER 1999
Title of Invention:	SYSTEM FOR INTRODUCING OPTICAL TWEEZERS AND/OR A TREATMENT BEAM INTO A MICROSCOPE *(Anordnung zur Einkopplung einer Optischen Pinzette und/oder eines Bearbeitungsstrahles in ein Mikroskop)*	
Applicant(s) for (DO/EO/US):	Ronald WENDENBURG, Anja HOFFMANN, Karl Otto GREULICH, Shamei MONAJEMBASHI and Volker UHL	

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

[X] 1. This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
[ ] 2. This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
[ ] 3. This express request to begin national examination procedures [35 U.S.C. 371 (f)] at any time rather than delay examination until the expiration of the applicable time limit set forth in 35 U.S.C. 371(b) and PCT Articles 22 and
[ ] 4. A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
[X] 5. A copy of the International Application as filed [35 U.S.C. 371(c)(2)]
  a) __ is transmitted herewith (required only if not transmitted by the International Bureau)
  b) __ has been transmitted by the international Bureau
  c) __ is not required, as the application was filed in the United States Receiving Office (RO/US)
[ ] 6. A translation of the International Application into English [35 U.S.C. 371(c)(2)] ***TO FOLLOW***
[ ] 7. Amendments to the claims of the International Application under PCT Article 19 [35 U.S.C. 371(c)(3)]
  a) __ are transmitted herewith (required only if not transmitted by the International Bureau)
  b) __ have been transmitted by the International Bureau
  c) __ have not been made; however, the time limit for making such amendments has **NOT** expired.
  d) __ have not been made and will not be made
[ ] 8. A translation of the amendments to the claims under PCT Article 19 [35 U.S.C. 371(c)(3)]
[ ] 9. An Oath or declaration of the inventor(s) [35 U.S.C. 371(c)(4)] **Executed Decl/POA TO FOLLOW**
[ ] 10. A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 [35 U.S.C. 371(c)(5)]

Items 11. to 16. Below concern other document(s) or information included:

[X] 11. An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98
[ ] 12. An Assignment document for recording. A separate cover sheet (PTO-1619A) in compliance with 37 CFR 3.28 and 3.31 is included.
[ ] 13. __ A **FIRST** preliminary amendment
  __ A **SECOND** or **SUBSEQUENT** preliminary amendment
[ ] 14. A **substitute specification**
[ ] 15. A change of power of attorney and/or address letter
[X] 16. (other items or information) **PCT/RO/101, WO 01/35150 dated 17MAY01, Search Report (PCT/ISA/220 and 210) dated 9FEB01, German Examination Report dated 3MAR00 and PTO-1449 w/11 references (including 4 English Abstract(s))**

EXPRESS MAIL No.: **EL 915 669 343 US** Deposited: **July 9, 2001**
I hereby certify that this correspondence is being deposited with the United States Postal Service Express mail under 37 CFR 1.10 on the date indicated above and is addressed to: BOX PCT, Commissioner for Patents, Washington, DC 20231.

/Ruth Montalvo Date: July 9, 2001

09/869951

JC18 Rec'd PCT/PTO 09 JUL 2001

		CALCULATIONS	PTO USE ONLY
[X] 17. The following fees are submitted: BASIC NATIONAL FEE [37 CFR 1.492(a)(1)-(5)]			
[X] Search Report has been prepared by the EPO or JPO.....	$ 860.00		
[ ] International preliminary examination fee paid to USPTO [37 CFR 1.482].....	$ 690.00		
[ ] No International preliminary examination fee paid to USPTO [37 CFR 1.482] but International search fee paid to USPTO [37 CFR 1.445(a)(2).....	$ 710.00		
[ ] Neither International preliminary examination fee [37 CFR 1.482] nor International search fee [37 CFR 1.445(a)(2)] paid to USPTO.....	$ 1,000.00		
[ ] International preliminary examination fee paid to USPTO [37 CFR 1.482] and all claims satisfied provisions of PCT Article 33(1)-(4).....	$ 100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT:		**$860.00**	

Claims	Number Filed		Number Extra	Rate	
Total Claims	6	-20		x $ 18. =	
Indep. Claims	1	-03		x $ 80. =	
[__] Multiple Dependent Claim(s) (if applicable)				+ $ 270. =	

TOTAL OF ABOVE CALCULATIONS:	**$860.00**	
Surcharge of $130.00 for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date [37 CFR 1.492(e)] ***TO FOLLOW***		
TOTAL OF ABOVE CALCULATIONS:	**$860.00**	
Applicant claims Small Entity Status [See 37 CFR 1.27] Reduction by ½ for filing by small entity		
SUBTOTAL:	**$860.00**	
Processing fee of $130.00 for furnishing the English Translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date [37 CFR 1.492(f)]	**$130.00**	
TOTAL NATIONAL FEE:	**$990.00**	
Fee for recording the enclosed assignment [37 CFR 1.21(h)] The assignment must be accompanied by an appropriate cover sheet (PTO-1595) [37 CFR 3.28, 3.31].$ 40.00 per property +		
TOTAL FEE(S):	**$990.00**	
AMOUNTS TO BE REFUNDED OR CHARGED	**REFUNDED** $ **CHARGED** $	

~~(Please note the filing fee is based on the claims in the Preliminary Amendment)~~

[X] Check in the amount of **$ 990.00** to cover the above fees is enclosed. (The Commissioner is hereby authorized to charge any additional fees required with this submission or to credit any overpayment to Deposit Account No: 50-1529.)

NOTE: Where an appropriate time limit under 36 CFR 1.494 or 1.495 has not been met, a petition to revive [37 CFR 1 137(a) or (b)] must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Gerald h. Kiel, Esq. **(Customer No. 026418)**
Reed Smith LLP
375 Park Avenue
New York, NY 10152

Gerald H. Kiel — Gerald H Kiel — 25,116 — July 9, 2001

Name (Tel. (212) 521-5400) — Signature — Reg. No. — Date

EXPRESS MAIL mailing label No. **EL 645 877 386 US** Date of Deposit **December 10, 2001**

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner for Patents, Washington, D.C. 20231.

Ruth Montalvo — Date: 10 Dec

Docket No.:GK-ZEI-3130/500343.20130

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Ronald WENDENBURG, Anja HOFFMANN, Karl Otto GREULICH, Shamci MONAJEMBASHI and Volker UHL
Serial No.:	09/869,951
Filed:	July 9, 2001
For:	SYSTEM FOR INTRODUCING OPTICAL TWEEZERS AND/OR A TREATMENT BEAM INTO A MICROSCOPE

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to receipt of a first Office Action, please amend the above-identified application as follows:

IN THE SPECIFICATION

Cancel the present specification and substitute therefor the enclosed substitute specification.

IN THE CLAIMS

Page 7, line 1, change "Patent Claims" to –What is claimed is--.

Cancel claims 1-6 and add new claims 7-12, reading as follows:

7. (New) In an arrangement for coupling at least one beam of optical tweezers for trapping particles and/or a treatment beam into a microscope beam path, preferably in a laser scanning microscope, comprising means for changing the position of the beam focus of the optical tweezers and/or of the treatment beam in a freely adjustable manner for purposes of changing the focal position of the microscope.

8. (New) The arrangement according to claim 7, wherein separate movable optics are provided for changing the position of the beam focus.

9 (New) The arrangement according to claim 7, wherein the beam outlet and/or illumination optics of the optical tweezers and/or of the treatment beam are/is displaceable in the direction of the optical axis.

10. (New) The arrangement according to claim 7, wherein the change is controllable and causes a movement of the optical tweezers and/or of the treatment beam in the direction opposite to the movement of the microscope objective.

11. (New) The arrangement according to claim 7, with a defined control of the displacement by previously stored or calculated values depending on the focal position.

12. (New) The arrangement according to claim 7, wherein there is provided a plurality of optical tweezers and/or treatment beams which are adjustable individually and/or jointly with respect to their focal position.

IN THE ABSTRACT OF THE DISCLOSURE

Cancel the present Abstract of the Disclosure and substitute therefor the enclosed Abstract of the Disclosure which is attached to the substitute specification..

REMARKS

Claims 1-6 have been cancelled and new claims 7-12 have been added. The amendments to the claims have been made only to improve the form of the claims for examination purposes.

The specification and abstract have been amended to conform it to U.S. format.

An early and favorable action on the merits is respectfully requested.

Respectfully submitted,

By: _____
Gerald H. Kiel
Reg. No. 25,116

December 10, 2001
REED SMITH LLP
375 Park Avenue
New York, NY 10152-1799
GHK:jl
Enc.: Substitute Specification
Abstract of the Disclosure

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Customer No.	026418	
Attorney's Docket No.:	GK-ZEI-3130 / 500343.20130	
U.S. Application No.:	09/869,951	
International Application No.:	PCT/EP00/10808	
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Applicant(s) for (DO/EO/US):	Ronald WENDENBURG, Anja HOFFMANN, Karl Otto GREULICH, Shamei MONAJEMBASHI and Volker UHL	

SUBSTITUTE SPECIFICATION and ABSTRACT

Docket No.: GK-ZEI-3130/500343.20130

SYSTEM FOR INTRODUCING OPTICAL TWEEZERS AND/OR A TREATMENT BEAM INTO A MICROSCOPE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority of PCT application Serial No. PCT/EP00/10808, filed November 2, 2000 and German application Serial No. 199 54 933.8, filed November 10, 1999, the complete disclosures of which are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

a) Field of the Invention

The invention enables spatial fixation of microscopic objects in laser scanning microscopes, also during the displacement of the object plane, for example, when recording an image. Therefore, moving objects can also be imaged sharply.

b) Related Background

Optical tweezers have proven to be an important work tool for a range of biological work techniques. Expanded experimental possibilities can be anticipated due to the combination of laser scanning microscopes with laser micro-techniques.

LSM recordings of moving objects, above all in the interior of unopened cells, often do not result in satisfactory images because many subcellular structures move during the scanning time. Ideally, the optical tweezers can be used for careful (vital) fixation. Further, a spatially defined displacement of fixed objects is possible with the optical tweezers. Application examples for the use of compensated optical tweezers in the laser scanning microscope are the examination of organelles, for example, chloroplasts, or the determination of objects which are moved by motor proteins. In the latter case, it is even possible under suitable

conditions to take energy measurements. In principle, moving objects, for example, particles in suspension or determined organelles, can not be imaged sharply without being fixated by compensated optical tweezers.

Optical tweezers which are coupled in through the objective have their focus in the object plane. When there is a parallel displacement of the object plane due to the three-dimensional image recording process (scanning), the focus of the optical tweezers is also displaced. As a result, objects that are held by the optical tweezers are likewise displaced. However, this is undesirable during image recording. Therefore, the displacement of the object plane must be compensated by a suitable device in the beam path of the optical tweezers.

One aspect of the present invention is to fulfill a need in laser scanning microscopes of different manufacturers whenever the optical tweezers are coupled in through the objective and the third dimension is made accessible during image recording by the displacement of the objective or object stage or by another method which displaces the focus of the optical tweezers relative to the specimen.

When optical tweezers are coupled into an inverted microscope via a second high-aperture objective which couples in the optical tweezers from the other side of the specimen (K. Visscher, G. J. Brakenhoff, "Single Beam Optical Trapping Integrated in a Confocal Microscope for Biological Applications", Cytometry 12:486-491)), a compensated movement of the optical tweezers is rendered superfluous. However, the specimen must be located between two glass coverslips for this purpose and may not exceed a certain thickness. Further, this type of in-coupling limits conventional microscope applications because the objective for the optical tweezers is placed at the location of the transmitted light beam path in the inverted microscope. Moreover, there is no longer unlimited free access to the specimen from above, which makes applications with microinjection devices or temperature regulating devices, for example, very difficult, if not impossible. This is also true for constructions in which optical fixation of particles is carried out by glass fibers which are provided with microlenses and which are guided directly onto the specimen. In addition, problems arise with respect to the sterility of the specimen because the glass fibers must be immersed in thicker liquid layers when

particles are to be fixated on the underside of the liquid. An aspect of the invention is to address these problems as well.

SUMMARY OF THE INVENTION

In accordance with the invention, an arrangement for coupling at least one beam of optical tweezers for trapping particles and/or a treatment beam with a microscope beam path, preferably in a laser scanning microscope comprising means for changing the position of the beam focus of the optical tweezers and/or of the treatment beam in a freely adjustable manner for purposes of changing the focal position of the microscope.

BRIEF DESCRIPTION OF THE DRAWINGS

In the drawings:

Figure 1 is a schematic illustration of the operation of the invention; and

Figure 2 shows application in a microscope such as a laser scanning microscope.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

In the construction which is relevant for the invention, the optical tweezers are guided through the microscope objective into the object plane. They are adjusted in such a way that microscopic particles located in the object plane are fixed, that is, the focus of the optical tweezers lies in the object plane. However, in three-dimensional image recording through a laser scanning microscope, the object plane must be displaced in parallel in order to access the third dimension projecting out of the object plane. Accordingly, the focus of the optical tweezers is also displaced, which leads to an unwanted displacement of the fixated particles. Three-dimensional objects that are held by the optical tweezers can not be recorded in three-dimensional resolution without compensating for this displacement. Compensation of the displacement of the object plane, hereinafter referred to as z-compensation, comprises variable optical elements which are inserted into the beam

path of the optical tweezers and compensate for the movement of the object plane. The z-compensation causes a compensating movement of the optical tweezers which takes place simultaneous with the movement of the object plane, so that the position of the fixated object in the specimen is maintained.

The compensation is achieved via an electromechanically displaceable optical element in the in-coupling system of the optical tweezers. The exact position of the object plane is taken from the control electronics of the laser scanning microscope during the image recording process. Correspondingly, the displaceable optical element in the in-coupling system of the optical tweezers is moved in a computer-controlled manner so that the position of the fixated object relative to the specimen is maintained. In principle, it is not necessary to determine the position of the object plane from the control electronics of the laser scanning microscope since the position of the relevant optical elements can also be detected electromechanically or optically. However, this entails high expenditure.

When the beam path for the optical tweezers from the laser to the microscope is effected via light guides, the z-compensation can be combined with the microscope-side arrangement for holding the light guide. This results in a compact unit with a minimum of optical elements.

It is also possible to carry out the z-compensation manually. For this purpose, the object to be examined is scanned in different x-y sections. The object plane is displaced between the sections by the laser scanning microscope. Before recording the next section, the position of the focus of the optical tweezers is returned to the starting point by manual displacement of the additional optical element located in the beam path of the optical tweezers. This process is repeated for every x-y section. However, the computer-controlled electromechanical displacement of the compensation element described above saves times during the three-dimensional image recording, which can be of decisive importance in case of ephemeral preparations. However, the z-compensation, per se, is to be patented independent from its technical application.

If several objects moving in liquid are to be examined, all of them must be fixated by optical tweezers. The z-compensation described herein also

allows the coupling in of a multitrap, as it is called, of optical tweezers in which one or more laser beams are directed to a plurality of objects for fixating. This can also be carried out in that a beam is directed alternately to a plurality of objects by means of a scanner mirror at high frequency in such a way that these objects remain fixated even when the laser beam does not permanently irradiate the corresponding object.

In the same way as the optical tweezers, a laser microbeam can also be coupled in so as to be compensated (a laser microbeam is a short pulse laser beam which is coupled into a microscope for purposes of micro-material treatment). Accordingly, the same optics as those used for the optical tweezers can be used for coupling in the laser microbeam. A z-compensated laser microbeam allows precise material treatment during image recording, for example, in order to examine in detail the interaction between light and material.

Figure 2 shows a microscope beam path with a specimen P, an objective O and a tube lens TL. By means of a deflecting mirror US, a laser beam L1 which scans the specimen P in x/y direction is coupled in via a scan lens SL, x/y scanner SC, deflecting mirror US1 and dichroic beam splitter ST1.

Vertical adjustment of the beam focus in the specimen is carried out by displacing the objective O in Z-direction via a control unit AS, so that the specimen can be scanned at different Z-positions.

The beam coming from the specimen reaches a detector unit comprising pinhole optics PO, pinhole PH and detector DE on a reverse path via the beam splitter ST1.

Further, an HBO illumination can be coupled in via another beam splitter ST2 and a lens L.

Further, in a variant V1, a pulsed laser beam L2 for optical cutting and another laser beam L3 as optical tweezers are coupled in through corresponding correcting optics O1, O2 via the beam splitter ST2 and another beam splitter ST3.

Light can be coupled in, for example, by indirect in-coupling via lightguides followed by collimating optics. The beam focus position of the respective laser in the specimen P is changed by displacing the optics or the ends of the light guides along the optical axis. In variant V1, the correcting optics O1, O2

are arranged so as to be displaceable along the optical axis via the control unit AS, wherein the control unit AS can match this displacing movement to the displacing movement of the objective. This is effected by means of an oppositely directed movement of at least one laser beam L3, which movement is adapted to the displacement of the objective via calculated or previously stored correction values. In this way, on the one hand, the position of the focus within the specimen can be changed in Z-direction in a defined manner; on the other hand, an object which is held by the optical tweezers can advantageously always remain at the same place in the specimen during displacements of the objective in Z-direction.

In addition to the movement of the optics O2 for laser L3, the optics O1 for the cutting laser L2 can also be moved in a corresponding manner and the position of the section can accordingly be selected in any manner, also so as to be decoupled from the position of the laser L3.

In the next variant V2, shared displaceable correcting optics O3 are provided for lasers L2, L3. In this case also, a decoupling of the movement of L2 and L3 can be achieved by means of different optics which can be additionally used in the beam path of laser L2.

Further, it is also possible to use multibeam tweezers, as they are called, i.e., tweezers in which one or more laser beams can be used to hold a plurality of objects. This can be carried out in that the laser beam L3 is directed to a plurality of objects by means of a scanner mirror at high frequency in such a way that these objects can be held simultaneously (C. Hoyer, S. Monajembashi, K. O. Greulich, "Laser Manipulation and UV induced single molecule reactions of individual DNA molecules", Journal of Biotechnology 52 (1996), 65-73).

Often organelles can not be imaged sharply because they move during image recording. Sharp three-dimensional images are possible only through the use of compensated optical tweezers which enable the organelles to be fixated while recording images. Thus, cell organelles, e.g., chloroplasts or mitochondria, can be fixated in living cells and imaged three-dimensionally in a sharp manner. Organelles which normally do not move, such as secretory vesicles or gravitational perception apparatus, can be deflected from their original position by the optical

tweezers and the reaction of the cells (reorganization) can be examined three-dimensionally. By deflecting from the rest position, the cytoskeletal dynamics in living cells can also be examined.

Spheroids can be manipulated and examined three-dimensionally as in vivo models for tissues with z-compensated optical tweezers in the laser scanning microscope.

Living cells can be stained by vital dyes in such a way that they can be imaged by fluorescence microscopy. Accordingly, it is possible to examine chromosome organization in living cells with z-compensated optical tweezers integrated in a confocal laser scanning microscope. Three-dimensional images and examinations for the separation process in nonadherent cells is also possible with this arrangement.

While the foregoing description and drawings represent the present invention, it will be obvious to those skilled in the art that various changes may be made therein without departing from the true spirit and scope of the present invention.

ABSTRACT OF THE DISCLOSURE

An arrangement for coupling at least one beam of optical tweezers for trapping particles and/or a treatment beam into a microscope beam path, preferably in a laser scanning microscope, wherein means are provided for changing the position of the beam focus of the optical tweezers and/or of the treatment beam in a freely adjustable manner for purposes of changing the focal position of the microscope.

500343.20130
GK-ZEI-3130

Title:

SYSTEM FOR INTRODUCING OPTICAL TWEEZERS AND/OR A TREATMENT BEAM INTO A MICROSCOPE

The invention enables spatial fixation of microscopic objects in laser scanning microscopes, also during the displacement of the object plane, for example, when recording an image. Therefore, moving objects can also be imaged sharply.

Background of the Invention

Optical tweezers have proven to be an important work tool for a range of biological work techniques. Expanded experimental possibilities can be anticipated due to the combination of laser scanning microscopes with laser micro-techniques.

LSM recordings of moving objects, above all in the interior of unopened cells, often do not result in satisfactory images because many subcellular structures move during the scanning time. Ideally, the optical tweezers can be used for careful (vital) fixation. Further, a spatially defined displacement of fixed objects is possible with the optical tweezers. Application examples for the use of compensated optical tweezers in the laser scanning microscope are the examination of organelles, for example, chloroplasts, or the determination of objects which are moved by motor proteins. In the latter case, it is even possible under suitable conditions to take energy measurements. In principle, moving objects, for example, particles in suspension or determined organelles, can not be imaged sharply without being fixated by compensated optical tweezers.

Optical tweezers which are coupled in through the objective have their focus in the object plane. When there is a parallel displacement of the object plane due to the three-dimensional image recording process (scanning), the focus of the optical tweezers is also displaced. As a result, objects that are held by the

optical tweezers are likewise displaced. However, this is undesirable during image recording. Therefore, the displacement of the object plane must be compensated by a suitable device in the beam path of the optical tweezers.

The invention is needed in laser scanning microscopes of different manufacturers whenever the optical tweezers are coupled in through the objective and the third dimension is made accessible during image recording by the displacement of the objective or object stage or by another method which displaces the focus of the optical tweezers relative to the specimen.

When optical tweezers are coupled into an inverted microscope via a second high-aperture objective which couples in the optical tweezers from the other side of the specimen (K. Visscher, G. J. Brakenhoff, "Single Beam Optical Trapping Integrated in a Confocal Microscope for Biological Applications", Cytometry 12:486-491)), a compensated movement of the optical tweezers is rendered superfluous. However, the specimen must be located between two glass coverslips for this purpose and may not exceed a certain thickness. Further, this type of in-coupling limits conventional microscope applications because the objective for the optical tweezers is placed at the location of the transmitted light beam path in the inverted microscope. Moreover, there is no longer unlimited free access to the specimen from above, which makes applications with microinjection devices or temperature regulating devices, for example, very difficult, if not impossible. This is also true for constructions in which optical fixation of particles is carried out by glass fibers which are provided with microlenses and which are guided directly onto the specimen. In addition, problems arise with respect to the sterility of the specimen because the glass fibers must be immersed in thicker liquid layers when particles are to be fixated on the underside of the liquid.

Description

Figure 1 is a schematic illustration of the operation of the invention;

Fig. 2 shows application in a microscope such as a laser scanning microscope.

In the construction which is relevant for the invention, the optical tweezers are guided through the microscope objective into the object plane. They are adjusted in such a way that microscopic particles located in the object plane are fixed, that is, the focus of the optical tweezers lies in the object plane. However, in three-dimensional image recording through a laser scanning microscope, the object plane must be displaced in parallel in order to access the third dimension projecting out of the object plane. Accordingly, the focus of the optical tweezers is also displaced, which leads to an unwanted displacement of the fixated particles. Three-dimensional objects that are held by the optical tweezers can not be recorded in three-dimensional resolution without compensating for this displacement. Compensation of the displacement of the object plane, hereinafter referred to as z-compensation, comprises variable optical elements which are inserted into the beam path of the optical tweezers and compensate for the movement of the object plane. The z-compensation causes a compensating movement of the optical tweezers which takes place simultaneous with the movement of the object plane, so that the position of the fixated object in the specimen is maintained.

The compensation is achieved via an electromechanically displaceable optical element in the in-coupling system of the optical tweezers. The exact position of the object plane is taken from the control electronics of the laser scanning microscope during the image recording process. Correspondingly, the displaceable optical element in the in-coupling system of the optical tweezers is moved in a computer-controlled manner so that the position of the fixated object relative to the specimen is maintained. In principle, it is not necessary to determine the position of the object plane from the control electronics of the laser scanning microscope since the position of the relevant optical elements can also be detected electromechanically or optically. However, this entails high expenditure.

When the beam path for the optical tweezers from the laser to the microscope is effected via light guides, the z-compensation can be combined with the microscope-side arrangement for holding the light guide. This results in a compact unit with a minimum of optical elements.

It is also possible to carry out the z-compensation manually. For this purpose, the object to be examined is scanned in different x-y sections. The object plane is displaced between the sections by the laser scanning microscope. Before recording the next section, the position of the focus of the optical tweezers is returned to the starting point by manual displacement of the additional optical element located in the beam path of the optical tweezers. This process is repeated for every x-y section. However, the computer-controlled electromechanical displacement of the compensation element described above saves times during the three-dimensional image recording, which can be of decisive importance in case of ephemeral preparations. However, the z-compensation, per se, is to be patented independent from its technical application.

If several objects moving in liquid are to be examined, all of them must be fixated by optical tweezers. The z-compensation described herein also allows the coupling in of a multitrap, as it is called, of optical tweezers in which one or more laser beams are directed to a plurality of objects for fixating. This can also be carried out in that a beam is directed alternately to a plurality of objects by means of a scanner mirror at high frequency in such a way that these objects remain fixated even when the laser beam does not permanently irradiate the corresponding object.

In the same way as the optical tweezers, a laser microbeam can also be coupled in so as to be compensated (a laser microbeam is a short pulse laser beam which is coupled into a microscope for purposes of micro-material treatment). Accordingly, the same optics as those used for the optical tweezers can be used for coupling in the laser microbeam. A z-compensated laser microbeam allows precise material treatment during image recording, for example, in order to examine in detail the interaction between light and material.

Figure 2 shows a microscope beam path with a specimen P, an objective O and a tube lens TL. By means of a deflecting mirror US, a laser beam L1 which scans the specimen P in x/y direction is coupled in via a scan lens SL, x/y scanner SC, deflecting mirror US1 and dichroic beam splitter ST1.

Vertical adjustment of the beam focus in the specimen is carried out by displacing the objective O in Z-direction via a control unit AS, so that the specimen can be scanned at different Z-positions.

The beam coming from the specimen reaches a detector unit comprising pinhole optics PO, pinhole PH and detector DE on a reverse path via the beam splitter ST1.

Further, an HBO illumination can be coupled in via another beam splitter ST2 and a lens L.

Further, in a variant V1, a pulsed laser beam L2 for optical cutting and another laser beam L3 as optical tweezers are coupled in through corresponding correcting optics O1, O2 via the beam splitter ST2 and another beam splitter ST3.

Light can be coupled in, for example, by indirect in-coupling via lightguides followed by collimating optics. The beam focus position of the respective laser in the specimen P is changed by displacing the optics or the ends of the light guides along the optical axis. In variant V1, the correcting optics O1, O2 are arranged so as to be displaceable along the optical axis via the control unit AS, wherein the control unit AS can match this displacing movement to the displacing movement of the objective. This is effected by means of an oppositely directed movement of at least one laser beam L3, which movement is adapted to the displacement of the objective via calculated or previously stored correction values. In this way, on the one hand, the position of the focus within the specimen can be changed in Z-direction in a defined manner; on the other hand, an object which is held by the optical tweezers can advantageously always remain at the same place in the specimen during displacements of the objective in Z-direction.

In addition to the movement of the optics O2 for laser L3, the optics O1 for the cutting laser L2 can also be moved in a corresponding manner and the position of the section can accordingly be selected in any manner, also so as to be decoupled from the position of the laser L3.

In the next variant V2, shared displaceable correcting optics O3 are provided for lasers L2, L3. In this case also, a decoupling of the movement of L2 and L3 can be achieved by means of different optics which can be additionally used in the beam path of laser L2.

Further, it is also possible to use multibeam tweezers, as they are called, i.e., tweezers in which one or more laser beams can be used to hold a plurality of objects. This can be carried out in that the laser beam L3 is directed to a

plurality of objects by means of a scanner mirror at high frequency in such a way that these objects can be held simultaneously (C. Hoyer, S. Monajembashi, K. O. Greulich, "Laser Manipulation and UV induced single molecule reactions of individual DNA molecules", Journal of Biotechnology 52 (1996), 65-73).

Application Examples

Often organelles can not be imaged sharply because they move during image recording. Sharp three-dimensional images are possible only through the use of compensated optical tweezers which enable the organelles to be fixated while recording images. Thus, cell organelles, e.g., chloroplasts or mitochondria, can be fixated in living cells and imaged three-dimensionally in a sharp manner. Organelles which normally do not move, such as secretory vesicles or gravitational perception apparatus, can be deflected from their original position by the optical tweezers and the reaction of the cells (reorganization) can be examined three-dimensionally. By deflecting from the rest position, the cytoskeletal dynamics in living cells can also be examined.

Spheroids can be manipulated and examined three-dimensionally as in vivo models for tissues with z-compensated optical tweezers in the laser scanning microscope.

Living cells can be stained by vital dyes in such a way that they can be imaged by fluorescence microscopy. Accordingly, it is possible to examine chromosome organization in living cells with z-compensated optical tweezers integrated in a confocal laser scanning microscope. Three-dimensional images and examinations for the separation process in nonadherent cells is also possible with this arrangement.

Patent Claims

1. Arrangement for coupling at least one beam of optical tweezers for trapping particles and/or a treatment beam into a microscope beam path, preferably in a laser scanning microscope, wherein means are provided for changing the position of the beam focus of the optical tweezers and/or of the treatment beam in a freely adjustable manner for purposes of changing the focal position of the microscope.

2. Arrangement according to claim 1, wherein separate movable optics are provided for changing the position of the beam focus.

3. Arrangement according to one of the preceding claims, wherein the beam outlet and/or illumination optics of the optical tweezers and/or of the treatment beam are/is displaceable in the direction of the optical axis.

4. Arrangement according to one of the preceding claims, wherein the change is controllable and causes a movement of the optical tweezers and/or of the treatment beam in the direction opposite to the movement of the microscope objective.

5. Arrangement according to one of the preceding claims, with a defined control of the displacement by means of previously stored or calculated values depending on the focal position.

6. Arrangement according to one of the preceding claims, wherein there is provided a plurality of optical tweezers and/or treatment beams which are adjustable individually and/or jointly with respect to their focal position.

Abstract

Arrangement for coupling at least one beam (L3) of optical tweezers for trapping particles and/or a treatment beam (L2) into a microscope beam path, preferably in a laser scanning microscope (O, SC), wherein means (AS) are provided for changing the position of the beam focus of the optical tweezers and/or of the treatment beam in a freely adjustable manner for purposes of changing the focal position of the microscope.

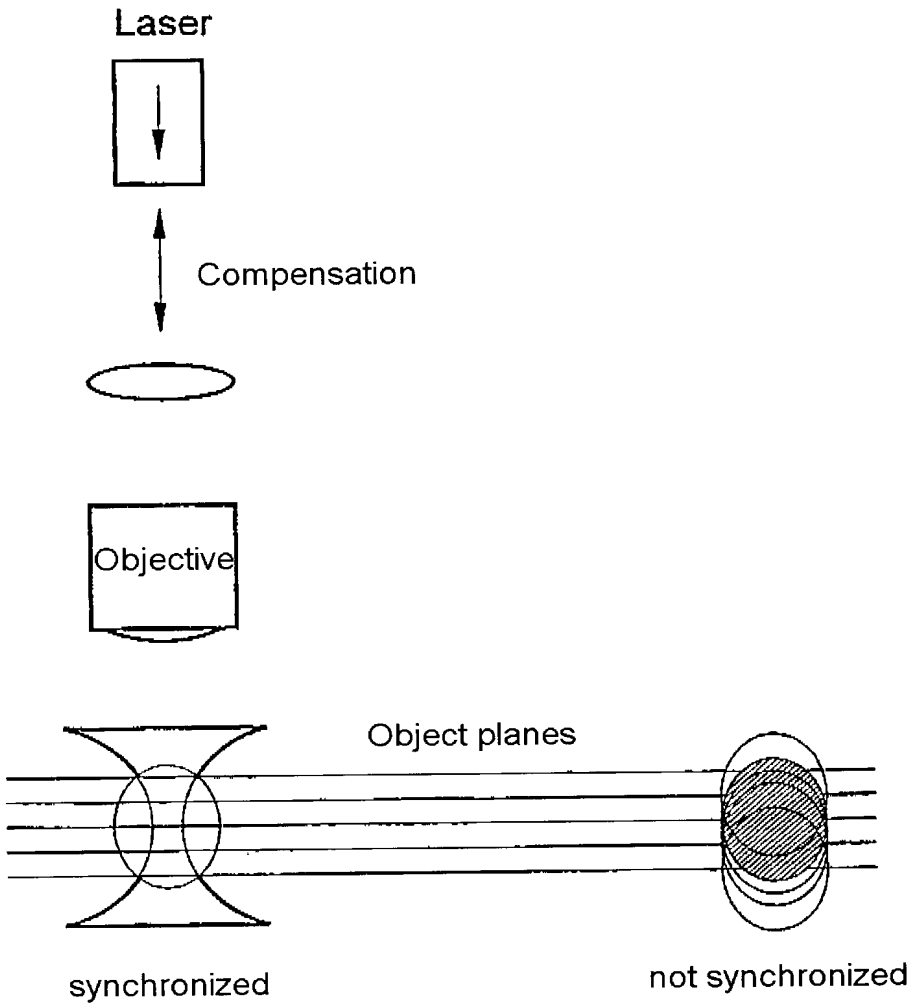
Laser
Compensation
Objective
Object planes
synchronized
not synchronized

Fig.1

2/2

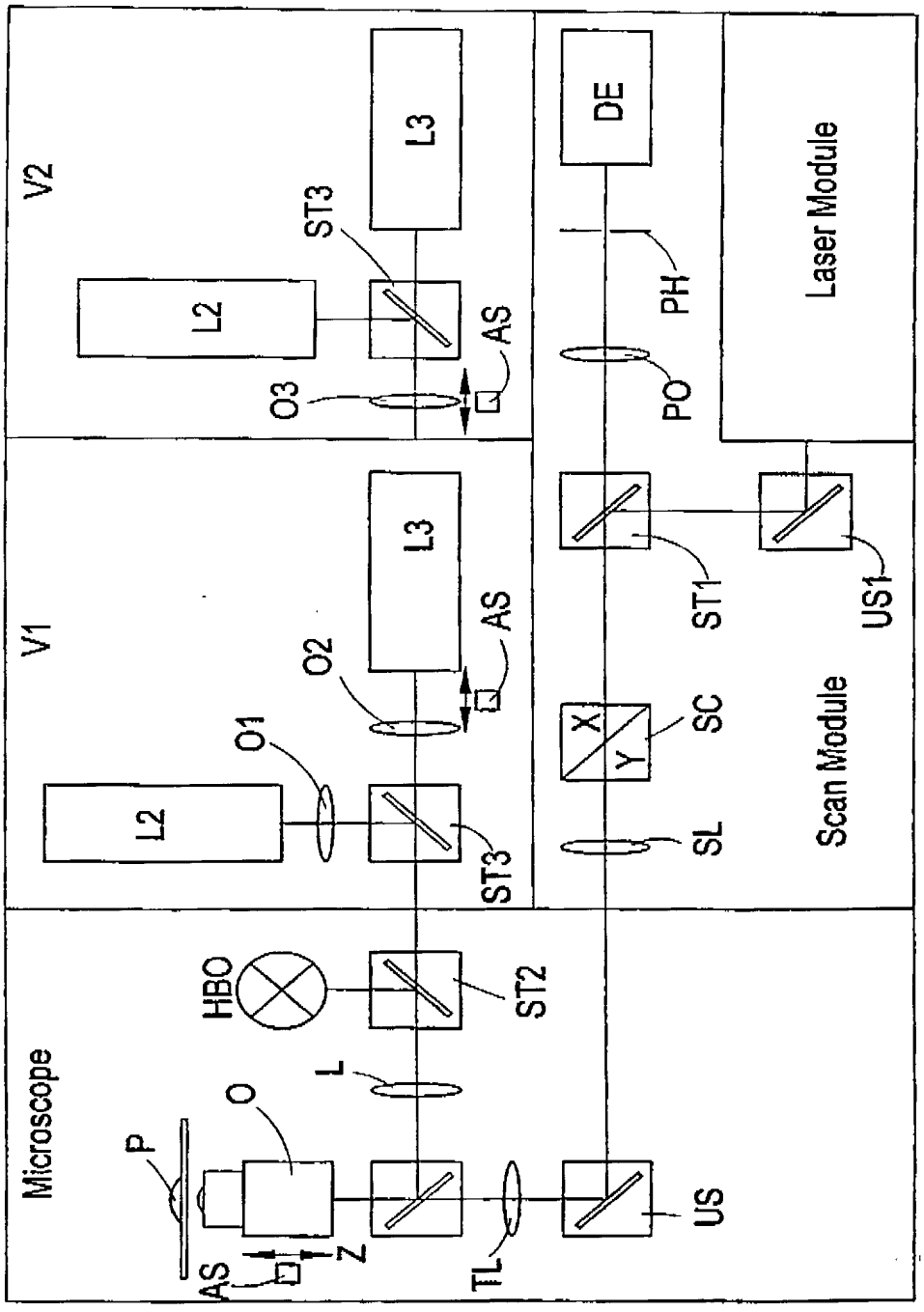
V2
L3
ST3
L2
AS
O3
DE
PH
PO
Laser Module
V1
L3
AS
O2
O1
L2
ST3
ST1
US1
X
Y
SC
SL
Scan Module
Microscope
HBO
ST2
L
O
P
AS
Z
TL
US

Fig.2

09869951 .121001

UNITED STATES OF AMERICA COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION	FILE NO. GK-ZEI-3130/ 500343.20130

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; that I verily believe that I am the original, first and sole inventor (if only one name is listed below) or a joint inventor (if plural inventors are named) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

SYSTEM FOR INTRODUCING OPTICAL TWEEZERS AND/OR A TREATMENT BEAM INTO A MICROSCOPE

The specification of which

☐ is attached hereto.

☒ was filed on July 9, 2001 as United States patent application Serial Number 09/869,951.

☒ was filed on November 2, 2000 as PCT international patent application No. PCT/EP00/10808 and was amended on _____ (if any).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information known to be material to patentability in accordance with Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)

COUNTRY	APPLICATION NUMBER	DATE OF FILING *(day, month, year)*	PRIORITY CLAIMED UNDER 35 U.S.C. § 119
Germany	199 54 933..8	10 November 1999	YES x NO ____
			YES ___ NO ____

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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